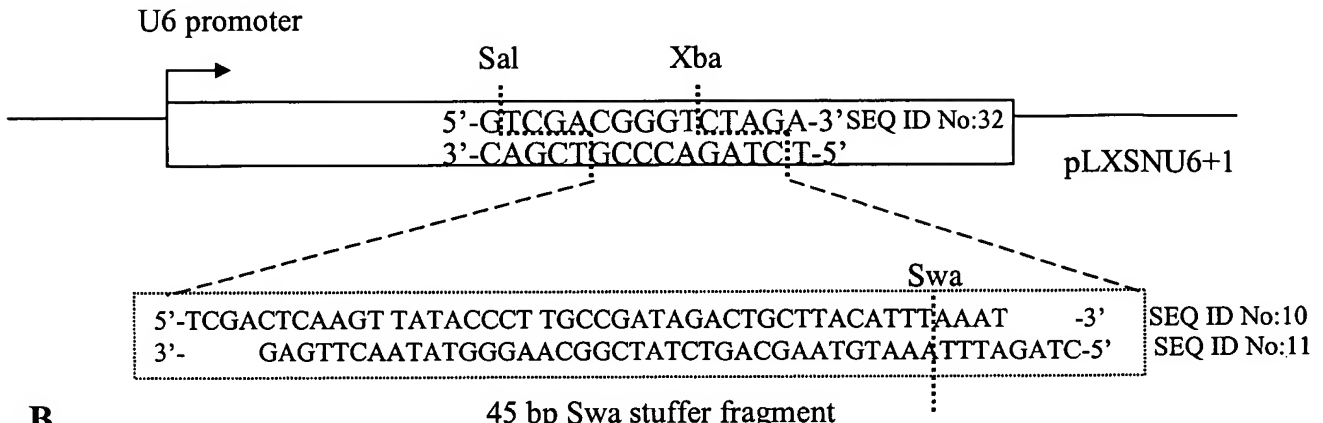


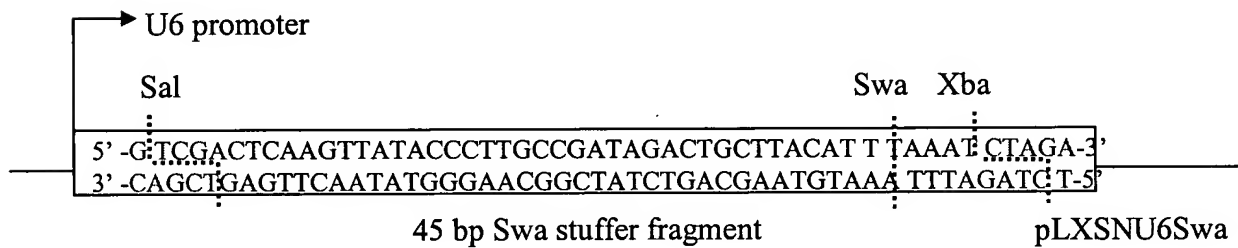
4/21

Figure 4. Construction of random shRNA expression library in a modified pLXSN retroviral vector

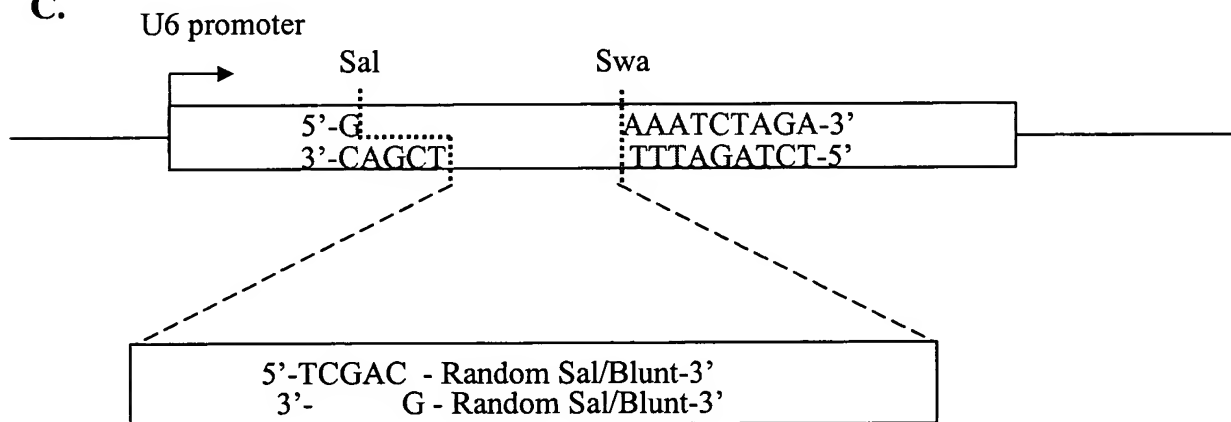
A.



B.



C.



BEST AVAILABLE COPY

5/21

Figure 5. Enzymatic generation of DNA insert encoding complementary sense and antisense RNAs specific for p53.

p53 siRNA Template (63 mer)

5' - CGGTGATTCCGTCGACCAAAAAGACTCCAGTGGTAATCTACTTTTTCTAGAGGTAACAGGCGC - 3' SEQ ID No:12

primer (17 mer)

5' - GCGCCTGTTACCTCTAG - 3' (SEQ ID NO:13)

1/ Anneal Primer to p53siRNA

5' - CGGTGATTCCGTCGACCAAAAAGACTCCAGTGGTAATCTACTTTTTCTAGAGGTAACAGGCGC - 3' SEQ ID No:12
3' - GATCTCCATTGTCCGCG - 5' SEQ ID No:13

2/ Second Strand Synthesis

5' - CGGTGATTCCGTCGACCAAAAAGACTCCAGTGGTAATCTACTTTTTCTAGAGGTAACAGGCGC - 3' SEQ ID No:12
3' - GCCACTAAGGGAGCTCGTTTTTCTGAGGTCACCATTAGATGAAAAAGATCTCCATTGTCCGCG - 5'

3/ SalI and XbaI Digestion

TCGACCAAAAAGACTCCAGTGGTAATCTACTTTTT
GGTTTTTCTGAGGTCACCATTAGATGAAAAAGATC

4/ Dephosphorylate and Purify

BEST AVAILABLE COPY

Figure 8. Construction of a genome-wide siRNA retroviral expression library.

A.

19N siRNA Template (63 mer)

5' -CGGTGATTCCCTCGAGCAAAAANNNNNNNNNNNNNNNNNNNNTTTTCTAGAGGTAACAGGCGC-3' SEQ ID No:18

19N primer (17 mer) 5' -GCGCCTGTTACCTCTAG-3' SEQ ID NO:13

1/ Anneal Primer to 19NsiRNA

5' - CCGTGATTCCCTCGAGCAAAAAANNNNNNNNNNNNNNNNNNTTTTCTAGAGGTAACAGGCGC-3', SEQ ID No:18
3' - GATCTCCATTGTCCGCG-5', SEQ ID No:13

2/ Second Strand Synthesis

5' - CGGTGATTCCCTCGAGCAAAAANNNNNNNNNNNNNNNNNNNNTTTTCTAGAGGTAACAGGCGC - 3' SEQ ID No:18
3' - GCCACTAAGGGAGCTCGTTTTNNNNNNNNNNNNNNNNNNNAAAAAGATCTCCATTGTCCGCG - 5'

3/ XhoI and XbaI Digestion

TCGACAAAAANNNNNNNNNNNNNNNNNNTTTT
GGTTTTNNNNNNNNNNNNNNNNNNAAAAAGATC

4/ Dephosphorylate and Purify

B.

